THE PROTEIN CONTENT OF HONEY

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Summary

A method is described for determining protein in honey. Removal of interfering materials of low molecular weight by dialysis allows the use of the Lowry photometric analysis for protein. For 740 samples of honey, a mean value of 169 mg/100 g was found, with a standard deviation of 71 mg/100 g and a range of 58-786. From 40% to 80% of the nitrogen in most honeys is in the protein fraction.

Introduction

It has been known for many years that honeys contain a small and variable amount of protein. Estimation of protein in honey by the volume of precipitate with tannin or phosphotungstic acid (Lund, 1909, 1910) or alcohol (Laxa, 1923) was used to distinguish between honey and artificial blends.

Relatively little attention has been given to honey proteins. Moreau (1911) confirmed their presence in honey with the common colour tests, and Stitz (1930) found peptones, albumins, some globulin, and some nucleo-protein. Paine et al. (1934) found over half of the colloidal material (removed by ultrafiltration) to be protein, and Mitchell et al. (1955), using trichloroacetic acid precipitation, reported that for 26 samples of heather (*Calluna vulgaris*) honey, the colloids ranged from about 58 to 74% protein, based on nitrogen content.

White and Kushnir (1967) found the proteins of 11 floral types of honey to consist of 4 to 7 components, and believed 4 components to originate with the bee. The approximate molecular weights of two of the proteins from the bee were about 40 000 and 240 000; other protein materials of plant origin had molecular weights about 98 000 and above 400 000. Bergner and Diemair (1975) found five proteins in honey, three of which were stated to originate with the bee.

Information on the composition of honey, particularly its variability, is useful in differentiating between genuine and adulterated honey. The availability of information on a larger number of components increases the probability of detection of fraud.

Advantage was taken of the existence of a collection of samples of nearly 500 United States honeys certified genuine by their producers; they had been obtained to develop methods for detecting the undeclared addition of high-fructose corn syrup to honey (White & Doner, 1978). These samples have been analysed for proline content (White & Rudyj, 1978).

In addition, 257 samples of United States honey that had been preserved in freezer storage were analysed. These comprise about half of the samples analysed in an earlier study by White et al. (1962), thus extending the value of those data.

Materials and Methods

1. A photometric procedure described by Bianchi (1976) was examined. Dilute honey is

treated with tungstic acid and centrifuged, and protein is determined on the dissolved precipitate by the biuret reaction.

2. A photometric procedure was developed in which amino acids and other interfering materials are removed from the sample by dialysis. DellaMonica et al. (1976) used dialysis similarly to eliminate materials interfering with the determination of available lysine. Total protein is determined on material not passing through the membrane (the tenate) by the method of Lowry et al. (1951).

Dialysis

Material

Regenerated cellulose dialysis tubing was used, with molecular cut-off of 12 000, $\frac{5}{8}$ inch (16 mm) diameter inflated, 1 inch (25 mm) width flat, from A. H. Thomas,* P.O. Box 779, Philadelphia, PA 19105, USA, catalog no. 3787 D-22 or equivalent.

Procedure

Instructions are as follows. Cut a 30-33 cm length of tubing, hydrate, and open under running tap water. (The possibility of chlorine from tap water supply having a slight effect on results (Saito et al., *Jap. J. exp. Med.* 43:523 (1973)), due to possible oxidation of tyrosine, has not been excluded.) Tie two knots at one end. Keep in water until used. Weight 5·00 g honey in small beaker, add 10 ml water, and stir to dissolve. Clip the open end of the dialysis tubing to the stem of a funnel, transfer the honey solution to the tubing, and rinse the beaker twice with a total of 10 ml water, adding this to the tubing. After excluding most of the air in the dialysis sac, tie two knots above the liquid, about 16·5 to 18 cm above the lower knots. Mix by inverting several times. Suspend in a container of running tap water, weighting the lower end if necessary. Dialyse for 16 h.

After dialysis, remove the sac from the container and hold it above a funnel leading to a dry 50-ml Erlenmeyer flask which has been weighed to 0·01 g. Pierce the lower end of the sac with a sharp instrument, so that the stream is directed into the funnel, and strip any residual liquid into the funnel with the fingers. Weigh the flask to obtain the volume (assume that the relative density is 1) of the tenate.

Protein determination

Reagents

A 2% Na_2CO_3 in 0·1-M NaOH

B_1 2% sodium potassium tartrate in water

B_2 1% $CuSO_4 \cdot 5H_2O$ in water

C Folin-Ciocalteu phenol reagent, 2N, diluted with an equal volume of water. (Concentrated reagent is available in pints as no. SO-P-24 from Fisher Scientific Co., 585 Alpha Drive, Pittsburgh, PA 15238, USA. Instructions for its preparation are given by Folin and Ciocalteu (1927).

Working reagent Add 0·50 ml solution B_1 and 0·50 ml B_2 (in that order) to 50 ml of solution A. Make fresh daily.

Procedure Pipette 0·50 ml tenate into each of three 18 × 150 ml testtubes, add 5·0 ml of the working reagent, mix well, and let stand at least 10 min. Then add 0·50 ml of Reagent C to each, mix well, and let stand for 1 h. Determine absorbance in 1-cm cell at 700 nm against a blank made with 0·50 ml water instead of tenate. Take the average of

* Reference to brand or firm does not constitute endorsement by the United States Department of Agriculture over others of a similar nature not mentioned.

the three values. Calibrate the method with 0·50 ml of undialysed solutions of crystalline egg albumin* containing up to 0·8 mg/ml protein.

Calculation Calibration factor = F = (mg/ml of standard)/(absorbance of standard). Absorbance of sample × F × wt tenate × 20 = mg protein/100 g honey.

Honey samples Two collections of United States honey samples are described by White and Rudyj (1978).

Results and Discussion

Comparison of methods

Although the precipitation procedure provided reasonably good recovery of protein (crystalline egg albumin*) added to honey (95·8%, 87·4%), lower values were obtained on honeys than those given by the dialysis method. By dialysing redissolved precipitates and the corresponding supernatant material, we found that 20% to 30% of the material retained and measured in the dialysis method escaped precipitation by tungstic acid under the specified conditions. We used the dialysis method for all work reported here.

Recovery of added protein and reproducibility

To two solutions, each containing 5·00 g of a honey sample (analysing 127 mg protein/100 g), were added 2·00 and 6·00 ml of a solution of crystalline egg albumin containing 1·00 mg/ml. The mixtures were found to contain 8·15 mg and 12·22 mg of protein by the dialysis procedure, corresponding to recoveries of 97·6% and 99·0%.

Eleven complete determinations (including dialysis and colour development and measurement) on the same sample were carried out, with simultaneous dialysis. The coefficient of variation (*cv*) was 1·4%. Two other honeys were analysed once each month for four months. The *cv* was 2·7% and 5·2% for samples averaging 65 and 206 mg/100 g, respectively.

The results of the analysis of the 740 honey samples are summarized in Table 1. The difference between the two groups is significant at the 0·05 probability level ($t = 2·12$, 738 df). Included in the 1974-75 samples are 10 honeydew honeys, identified by positive polarization. They do not differ in protein content from the other samples ($t = 0·75$, 738 df). The small difference in protein content between the two groups of samples is of little practical significance, and the values are combined in Fig. 1, which shows the distribution of all samples.

TABLE 1. Protein content of United States honeys.
(non-dialysable protein calculated as chicken egg albumin)

	1956-57	1974-75	All samples
Average (mg/100 g)	176·1	164·5	168·6
Standard deviation (mg/100 g)	84·4	62·3	70·9
Coefficient of variation (%)	47·9	37·9	42·1
Range (mg/100 g)	57·7-786	65·8-567	57·7-786
No. samples	257	483	740

Proline content is significantly ($r = 0·579$, $P < 0·001$) correlated with protein content. The magnitude of the correlation coefficient, not strikingly high, may be explained by the observations (White & Kushnir, 1967; Bergner & Diemair, 1975) that a portion of the protein originates in the plant, whereas all of the proline is believed to be added by the bees.

* Chicken egg albumin (ovalbumin) Grade V, essentially salt-free, crystallized and lyophilized; Sigma Chemical Co., St. Louis, MO, USA.

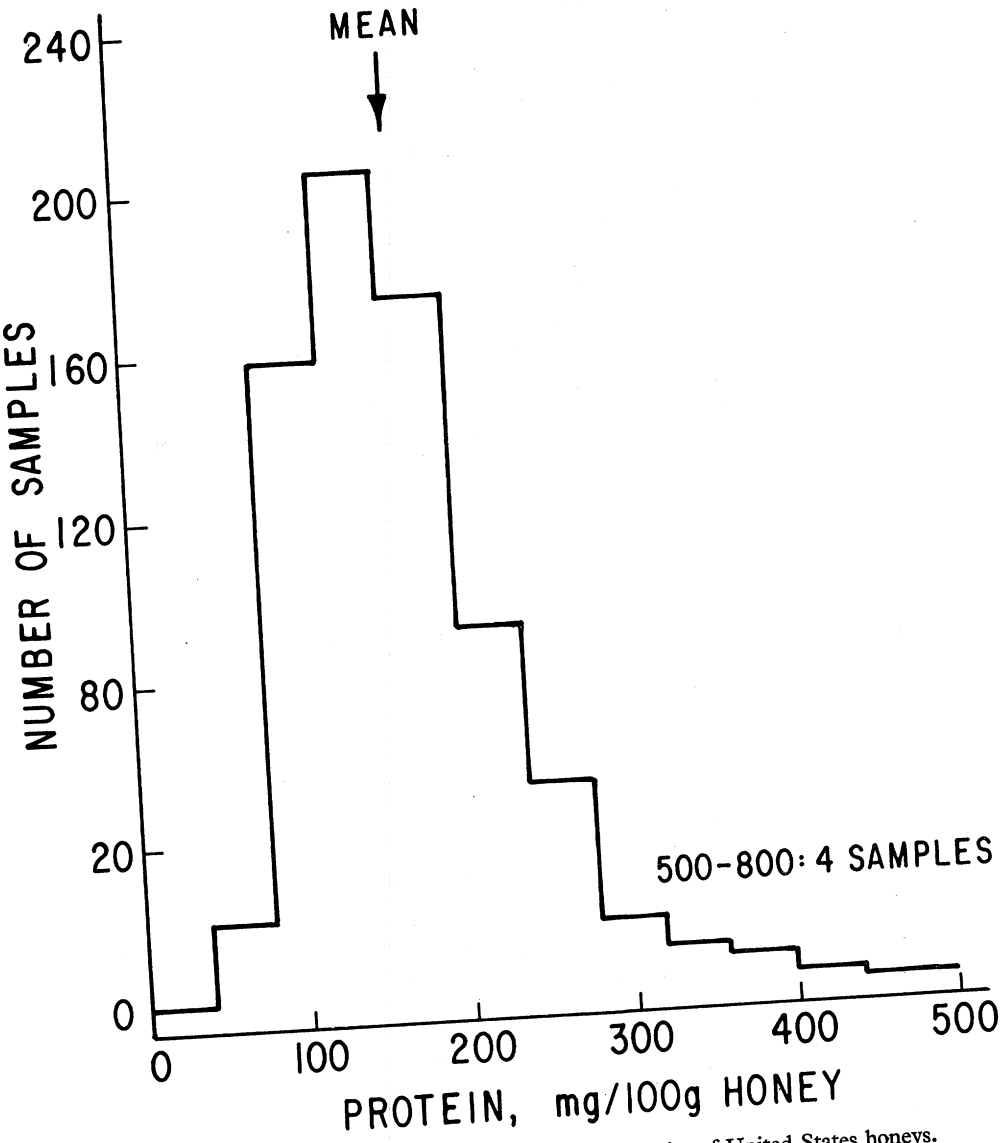

FIG. 1. Distribution of protein content among 740 samples of United States honeys.

The proportion of the total nitrogen in honey that is represented by protein (non-dialysable) and amino acids and small peptides, varies among samples. The availability of total nitrogen values for the 1956-57 samples (White et al., 1962) allowed the calculation of this proportion as (protein $\times$ 0·001)/(%N $\times$ 6·25). Table 2 shows the distribution of these values. A number of the samples with a calculated proportion greater than 1 had very low nitrogen contents; presumably these nitrogen values were considerably in error. They are not included in Table 2. In most honeys, protein represents 40-80% of the nitrogen content.

TABLE 2. Distribution of honey samples according to the proportion of the total nitrogen that was found in protein.

Proportion of nitrogen in protein	*Percentage of samples*
0·20-0·399	2·6
0·40-0·599	31·4
0·60-0·799	51·0
0·80-0·999	14·9

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